

Figure 1: Screening libraries of chimeric promoter sequences

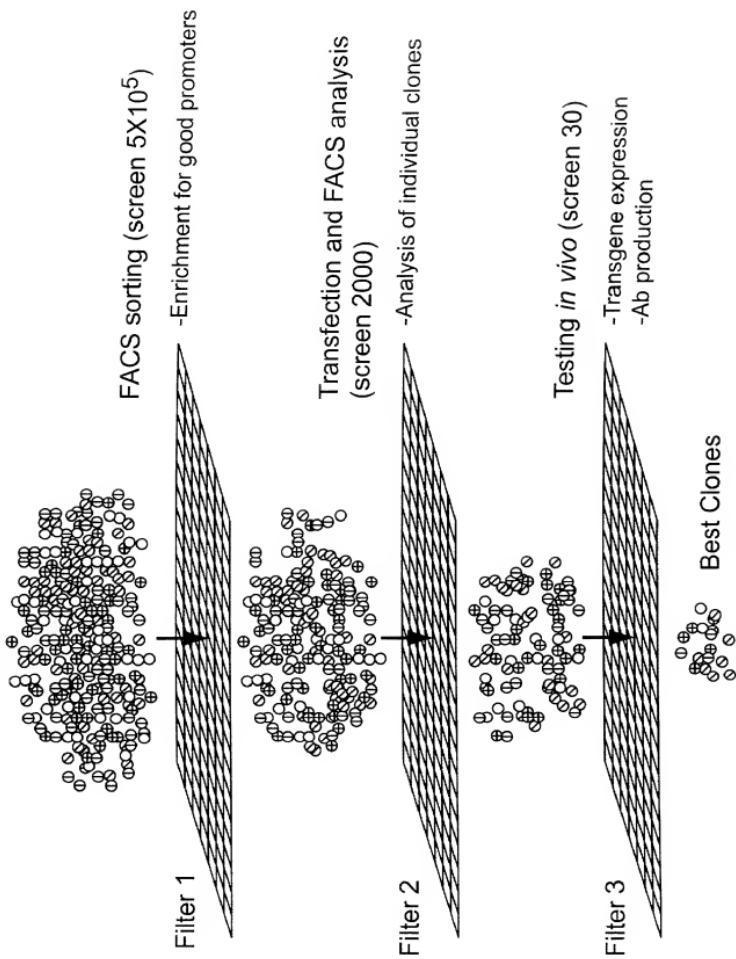


Figure 2: Enrichment of chimeric promoter libraries by FACS sorting

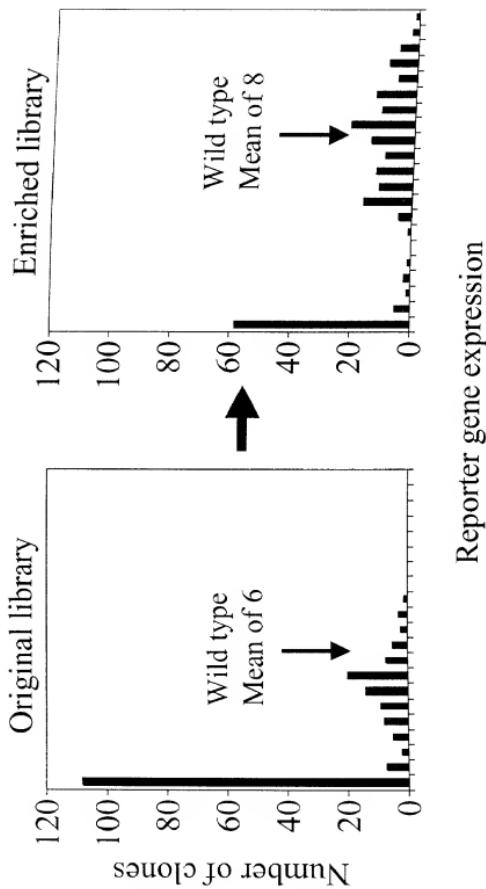


Figure 3: Diverse activities of chimeric promoter sequences in transfected cells

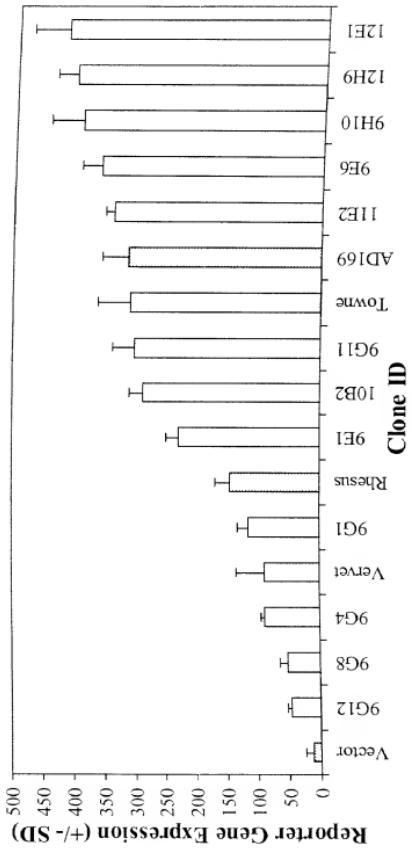


Figure 4: Luciferase expression in muscle 7 days after plasmid injection

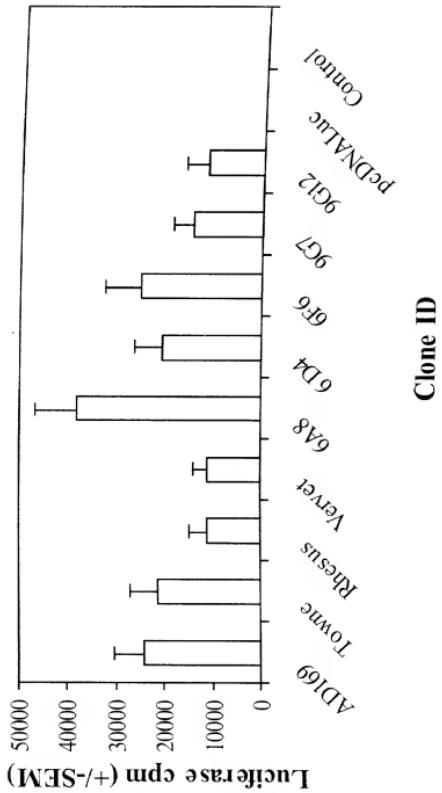


Figure 5: Comparison of Luciferase expression from clone  
6A8 and parental clones

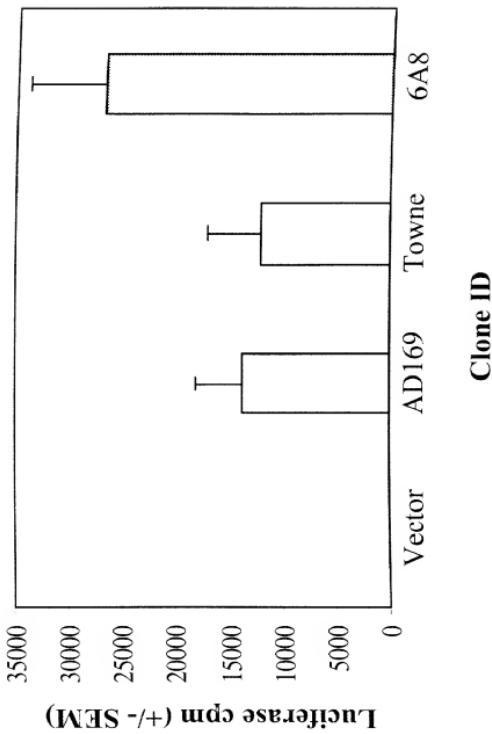
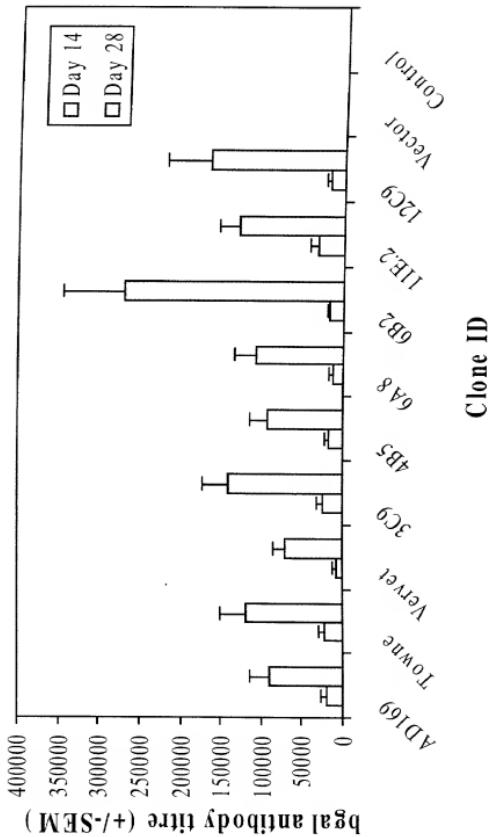
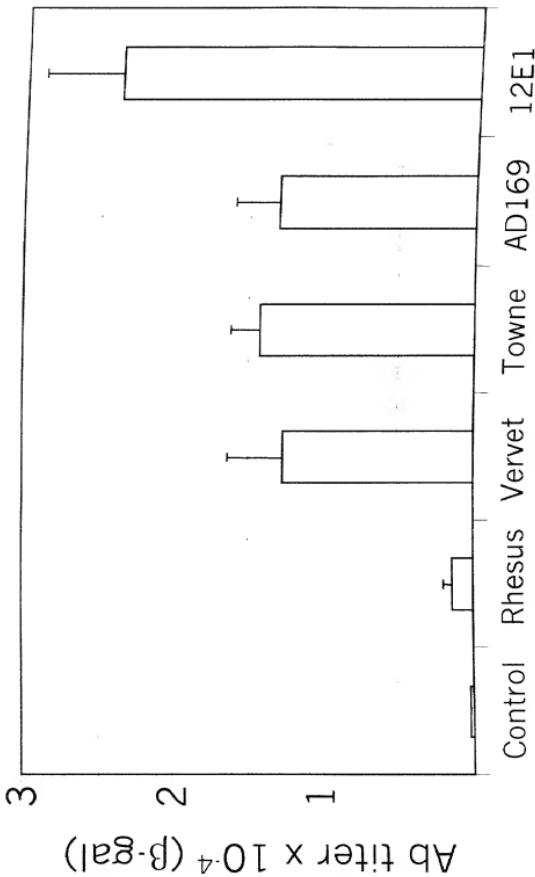


Figure 6A: Antibody responses following injection with  $\beta$ -galactosidase-encoding plasmid



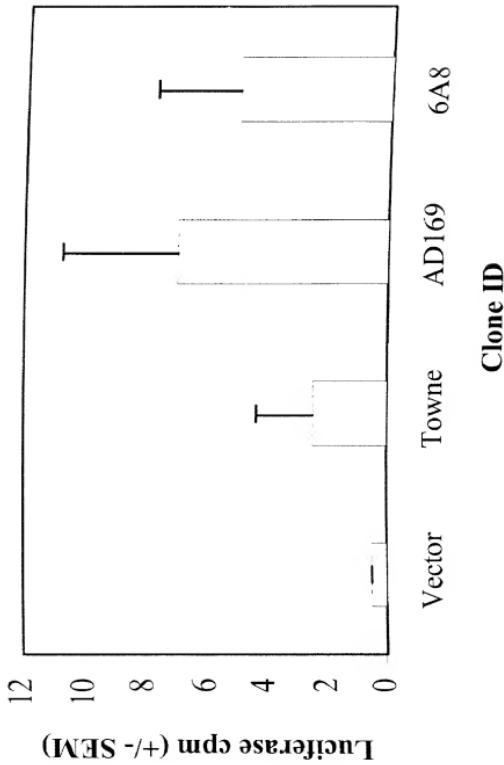
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Figure 6B: Improved Ab Response by Shuffled Promoter



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Figure 7: Chimeric promoter 6A8 is functional in human muscle tissue



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**Figure 8A:** Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD169 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.

Figure 8B: Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD169 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.

**Figure 8C:** Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD1 (69 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.

**Figure 8D:** Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD169 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.

Figure 8E: Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD169 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.



**Figure 8G:** Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD169 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.

	1.00	15/23	1.00
10B2	(1198) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
11E2	(1189) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
12C9	(898) -----		
12E1	(1147) TCCTGATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
12H9	(1198) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
3C9	(1198) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
4B5	(1146) TCCTGATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
6A8	(1198) TCCTGATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
6B2	(1146) TCCTGATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
6D4	(1146) TCCTGATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
6F6	(1188) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
9E1	(1100) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
9F11	(1196) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
9G12	(1198) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
9H4	(1198) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
9H7	(1188) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
9H8	(1198) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
AD169	(1198) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
Towne	(1197) TCGTATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG		
Consensus	(1201) TCCTGATTTTCAGATGGTGCCATTATTAAATTCACACCCGTCCCCGGTCCCGAGCTCCCGGTTTGTAAACATAGCTGG	1.400	
	1.301		
10B2	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
11E2	(1289) -----		
12C9	(898) -----		
12E1	(1247) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
12H9	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
3C9	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
4B5	(1246) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
6A8	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
6B2	(1246) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
6F6	(1288) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
9E1	(1105) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
9F11	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
9G12	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
9H4	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
9H7	(1288) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
9H8	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
AD169	(1298) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
Towne	(1297) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC		
Consensus	(1301) GATTCACGSGAAAATCGGTATGGTGCGAACATGGGTTCTCGTGTACGGGACTCTCACATCGACGCCGCTGGCTCATCTCGACGCGC	1.300	

**Figure 8H:** Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD169 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.

Figure 8t: Comparison of 18 chimeric promoter sequences generated by DNA shuffling using CMV promoter nucleic acid sequences from AD169 and Towne human strains and Rhesus and Vervet monkey strains as parental sequences.

Vector for promoter evolution

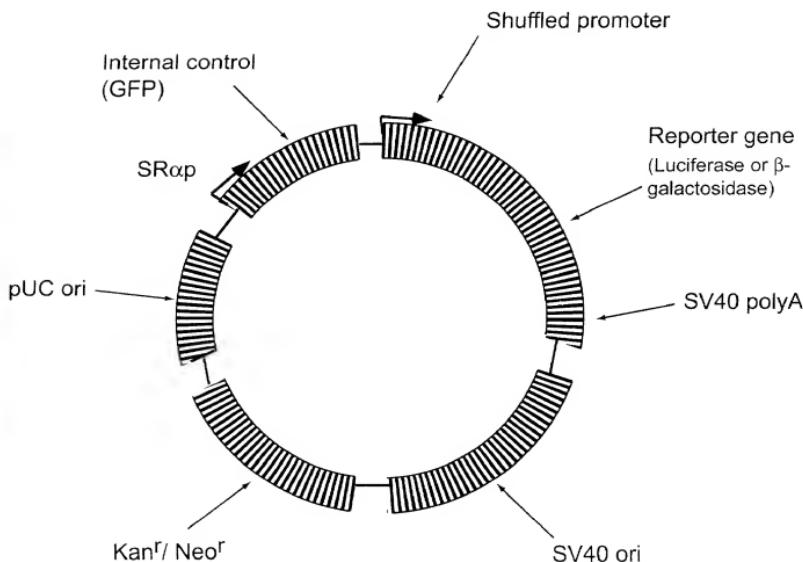


Fig. 9

**Figure 10A**

Towne_promoter_fr_PCR_prod_seq	ATA.....TGAGGCTTATACTGCCGATAGAGGGAGACATCAAGCTGGCACATGGCAATGCCAAATGCAT	60
Rhesus_monkey_PCR_prod_821bp	ACT....TGGCACGGTGCCAA.GTTTGGGGGGTC...TTGGPACGTGCCAA.....	
Vervet_(Simian)_PCR_product_seq	ATTGAATGGCATGGCCAATAATGGGGC..CATA...TTGGCTTATATGCGCA.....	
Towne_promoter_fr_PCR_prod_seq	ATCGATCTATACTATGAAATCAAATTGGCAATTAGCCATATTAGTCATTGGTTATATAGC	120
Rhesus_monkey_PCR_prod_821bp	...GTCCCGCCATATTGGTTGGAT....ATGTCATAATTATTGAT...CCATATAGC	
Vervet_(Simian)_PCR_product_seq	.....GGATCAATAT.....ATAGGCAATAATC.....ATAGGCAATAATC.....CAAATGGC'	
Towne_promoter_fr_PCR_prod_seq	ATAAATCAATAATGGCTATTGGCGATTGCAACCTTGTATCTATATGATAATATGTCAT	180
Rhesus_monkey_PCR_prod_821bp	CAATATCCAATAATGGCTAATAGCCA.....GGTCAATAGAATGGCCAATAGC	
Vervet_(Simian)_PCR_product_seq	CCTATGCCAATAATGGCTATTGGCCA.....GGTCAATAACTATGTTATGGCCCT	
Towne_promoter_fr_PCR_prod_seq	TATATATTGGCTCATGTCCAATATGACCGCCATGTGACATTGATTATCACTAGTT.AT	240
Rhesus_monkey_PCR_prod_821bp	CAATAT..GCCATGGCACAATGCAA.TGGGCCAGTATGATTATAGCCAATAT..AT	
Vervet_(Simian)_PCR_product_seq	ATGCCA..TATAGTATTCCATATGGGTTTCCTATGACGTAGATAGCCCCCTCCCAAT	

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Figure 10B

241 Towne\_promoter\_fr\_PCR\_prod\_seq TAATAGTA.....ATCAATTACGGGTCAATTAGTGTATGCCAATATGGAGTTCCGC  
Rhesus\_monkey\_PCR\_prod\_821bp AGGCAATA.....ATCCATATGG...CATATGTCCTATATGCCCTATGCCATTATGGC  
Vervet\_(Simian)\_PCR\_product\_seq GGGCGTCCCCATATACCATAATGG...GGCTTCCTAAATACCGCCATAGCCAATCCCCC

300 Towne\_promoter\_fr\_PCR\_prod\_seq GT...T..ACATAACTTACGGTAATGCCGCTGTGACCCGCCAACGACCCCCGGCC  
Rhesus\_monkey\_PCR\_prod\_821bp TTATGT..CCATTCAAAATTACCAATAATGGGTCTTCTTATATAGCTATAGGTACCGCC  
Vervet\_(Simian)\_PCR\_product\_seq AT...TGACGTCATGGTCTCTATATGGTCTTCTATTGAGCTATGGGGTCC

361 Towne\_promoter\_fr\_PCR\_prod\_seq .ATTGAGCT.....  
Rhesus\_monkey\_PCR\_prod\_821bp .ATTGAGCTTAATATGGATAACGGCTCCATTGACGTCATGGGAGGATAATATAAGTCAC  
Vervet\_(Simian)\_PCR\_product\_seq TATTGAGCTA.TATGGCCCTCCCCATTGACGTCATTACGGTAATGGCCCCTGGC

421 Towne\_promoter\_fr\_PCR\_prod\_seq TAATGACGGTATGTTCCCAT.....AGTAAACGCCATAGGG..ACTTTCCA  
Rhesus\_monkey\_PCR\_prod\_821bp TAATACGGCCATTGAGCTATAGGACCGTCCATTGACGTCATAGGGCCACCTTCCA  
Vervet\_(Simian)\_PCR\_product\_seq T..CAATGCCATTGACCT.....CAAATGGACCAACCA

480 Towne\_promoter\_fr\_PCR\_prod\_seq TAATGACGGTATGTTCCCAT.....AGTAAACGCCATAGGG..ACTTTCCA  
Rhesus\_monkey\_PCR\_prod\_821bp TAATACGGCCATTGAGCTATAGGACCGTCCATTGACGTCATAGGGCCACCTTCCA  
Vervet\_(Simian)\_PCR\_product\_seq T..CAATGCCATTGACCT.....CAAATGGACCAACCA

## Figure 10C

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481 Towne_promoter_fr_PCR_prod_seq TTGACGTCAATGGTGGAGTATTACGGTAAAATGCCCACTT.....GGCAATAC	540 Rhesus_monkey_PCR_prod_821bp TTGACGTCAATGG.....GTGCCCAATTGCCAATTC.....
541 Towne_promoter_fr_PCR_prod_seq ATCAAAGTGTATGATATGCCAAGTCCGGCCCTATTGAGGTAAATGACGGTAAATGCC	600 Rhesus_monkey_PCR_prod_821bp .....CCACGGCCCTATTGACGCTCAATGACGGTAAATGCC.
541 Vervet_(Simian)_PCR_product_seq .....TCACGGCCCTATTGACGCTCAATGACGGTAAATGCC.	
	660 Towne_promoter_fr_PCR_prod_seq GCCTGGCAATTAGCCCAACTACATGACCTTAACGGGACTTTCCTACTTGCGAGTACATC..T
	720 Rhesus_monkey_PCR_prod_821bp ACCTATTAATGTAACT ..TGGAAAGTAAATGGGTACTTGGCAATTACACCAAGG .TACAT
	Vervet_(Simian)_PCR_product_seq .....CACTTGGCAATTACAT
	661 Towne_promoter_fr_PCR_prod_seq ACGTATTAAGTCATCGCTATTACCATGGTGAATGCCAGTACACCAA.....
	Rhesus_monkey_PCR_prod_821bp ATCTATTAATGTAACT ..TGGCAAGTACATTACTATTGGCAATTACGCCAAGGGTACAT
	Vervet_(Simian)_PCR_product_seq

Figure 10D

721	.	.	.	
Towne_promoter_fr_PCR_prod_seq	.....TGGCGCTGATAGGT ..TTGACTCACGGGATTCGAAGTCTC			
Rhesus_monkey_PCR_prod_821bp	TGGCAG_TACGCCATTGACGTAAATGGCCGAAATGGCTGCCAAGTACA			
Vervet_(Simian)_PCR_product_seq	TGGCAGGTACTCCATTGACGTAAATGGCGGAAATGGCGCATGGCTGCCAAGTACA			
781	.	.	.	
Towne_promoter_fr_PCR_prod_seq	...CACCCCCATTGACGTCAATGGGAGTTGTITGGCACAAAATCAACGGGACTTTCGA			
Rhesus_monkey_PCR_prod_821bp	...TGCCCC_ATTGACGTCAATGGGG			
Vervet_(S-mian)_PCR_product_seq	ACATCCCC_ATTGACGTCAATGGGA.....			
841	.	.	.	
Towne_promoter_fr_PCR_prod_seq	AAATGTCGTAAATAACCCGCCCGTTGACCAAATGGGCC.....			
Rhesus_monkey_PCR_prod_821bp	.....CGGTCTPATGACGTCAAATGGGCC			
Vervet_(Simian)_PCR_product_seq	.....GGGCAATGACCCAAATGGCGTTCCATYGACCTAAATGGCG			
901	.	.	.	
Towne_promoter_fr_PCR_prod_seq	GTTAGGCCTTAACGGTGGAGGTCTATATAAGCAGACTCTGTTAGTAAACCGTCAAGATG			
Rhesus_monkey_PCR_prod_821bp	GTAGGGCTGC_CTAGGGCGGCTATATAACCAATSCACGTATGGGAACCGCATTCTG			
Vervet_(Simian)_PCR_product_seq	GTAGGGCTGCCTAATGGGACTCTATATAACCAATGCTGTTAGGGAAACCGCATTCTG			

**Figure 10E**

961 Towne_promoter_fr_PCR_prod_seq Rhesus_monkey_PCR_prod_821bp Vervet_(Simian)_PCR_product_seq	1020 CCTGGAGACGCATCCAGCTGTTGACCTCCAT_AGAAGAGAACCGGG.ACCGATCCAG CCTGGGGACGTG.....GAGGAGCACCATT.GAAAGGTACCGGGACCGATTCAG CCTGGGGACGTG.....GAGGAGCTCATTGAAAGAACCGGG.ACCGATTCAG	1021 Towne_promoter_fr_PCR_prod_seq Rhesus_monkey_PCR_prod_821bp Vervet_(Simian)_PCR_product_seq	1057 CCTCCGGGCCGGAAACCGTGGATTGGAAACGGCATT CCTCCCATAGCCGGAAAGGGTCAATTGGAAACGGCATA CCTCCCATAGCCGGGAUGGTCAATTGGAAATGGCATA
			SEQ ID NO : 20 SEQ ID NO : 22 SEQ ID NO : 23